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STUDIES ON REPRODUCTION IN THE HERMAPHRODITIC SEA STAR, *ASTERINA MINOR*: THE FUNCTIONAL MALE GONADS, "OVOTESTES."

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Abstract

Development of gonads in the sea star, *Asterina minor* Hayashi was studied by light microscopy. In adults, two series of gonads are found in one interradial area. Each series consists of an ovary, supernumerary ovaries, and ovotestes with a regular alignment to the margin. In breeding season, the ovotestes have sperm and some small basophilic oocytes. After releasing sperm, the ovotestes are much reduced in size, but the small oocytes remain. After spawning, as no rapid increase of testicular tissue occurs, these gonads resemble supernumerary ovaries. In addition to the previously existing series of gonads, a few new ovotestes are formed in more marginal positions annually. During and after the spawning period, the germinal cells in the genital rachis increase in number, differentiate into ovotestes, and form a gonoduct and gonopore of their own.

Hermaphrodism in this species is maintained by the annual formation and maturation of ovotestes and by the annual maturation of oocytes in ovaries (*cf.* Fig. 1).

Introduction

Most species in the genus *Asterina* are dioecious. However, hermaphroditic species have also been reported among *Asterina: Asterina scobinata* (Dartnall, 1970), and occasional hermaphrodites in *Asterina batheri* (Ohshima, 1929). In *Asterina gibbosa* sexual change occurs from male to female (Cuénot, 1898; Bacci, 1951; Delavault, 1966). These studies suggest the diversity related to sex in *Asterina.*

In the small sea star, *Asterina minor* (Hayashi, 1974), Komatsu *et al.* (1979) reported that hermaphrodism is the normal sexual condition and that the gonads consist of both ovarian and testicular tubules. However, the annual reproductive cycle is not yet known.

In the present study the morphology and histology of ovotestes including testicular tissue were examined in *Asterina minor* from Tokyo Bay through two reproductive cycles.

Materials and Methods

Specimens of *Asterina minor* (10 animals on the average) were collected approximately monthly for two years, from April 1979 to March 1981, from the intertidal zone of Hashirimizu, Kanagawa Prefecture, Japan. Animals ranged from

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Abbreviation: R, major radius (distance from the center of the disk to the tip of an arm).

TABLE I

The relationship between the size of animal (R) and the condition of the reproductive organs in Asterina minor *in April and May (1979, 1980).*

R* (mm)	Number of animals	Ovaries	Supernumerary ovaries	Ovotestes	Number of gonopores in each series of gonads
2.0–2.9	9	+	−	−	0
3.0–3.9	19	+	−	−	0–1
4.0–4.9	11	+	−	−	1
5.0–5.9	11	+ (M)	−	+ (M)	2–3
6.0–6.9	4	+ (M)	−	+ (M)	3–4
7.0–7.9	6	+ (M)	− or +	+ (M)	3–6
8.0–11.5	8	+ (M)	+	+ (M)	4–9

Presence of gonads and maturation were designated as follows: −, absent; +, present; M, mature sexual cells.

*R = major radius.

2.3 mm to 11.5 mm in major radius, the distance from the center of the disk to the tip of an arm.

The animals were fixed with 10 per cent formalin in sea water for a week, followed by decalcification with 10 per cent trichloroacetic acid for another week at 4°C. After dehydration in a graded ethanol series and embedding in paraffin, the whole bodies of the animals were sectioned serially 5 to 7 μm thick. Horizontal and transverse sections were stained with hematoxylin and eosin.

RESULTS

Three types of gonads are present in *Asterina minor*: ovary, supernumerary ovary, and ovotestis. The ovary is the largest gonad and contains oocytes all year round. Supernumerary ovaries are much smaller than the ovary and always contain young or mature oocytes. The ovotestes, the main target of this paper, are hermaphroditic gonads having testicular tissue and oocytes or, in a small number of cases, testicular tissue only. The three types of gonads align to form a series, and two series are found in each interradial region.

Correlations between body size and gonads

During April and May (the breeding season of *Asterina minor* in Tokyo Bay) when the gonads are sharply distinguishable, the maturity of gonads was compared with the measurements of the body size, namely major radius (R) in Table I. Almost all individuals larger than 5 mm in R are hermaphrodites whose gonads consist of ovotestes and ovaries; sperm and young oocytes in the former, large eosinophilic maturing oocytes in the latter. For each series of gonads, two or more gonopores are found in the oral side of the disk.

Individuals larger than 5 mm are adults with mature oocytes and sperm. Most individuals under 5 mm in R are immature sea stars having only one immature ovary for each series, and individuals 3.5–5 mm in R have one gonopore.

Three types of gonads in breeding season

The centrally located ovary expands into one or more branched tufts protruding into the coelomic cavity (Figs. 1, 2A, B). A gonoduct connects with the orifice of

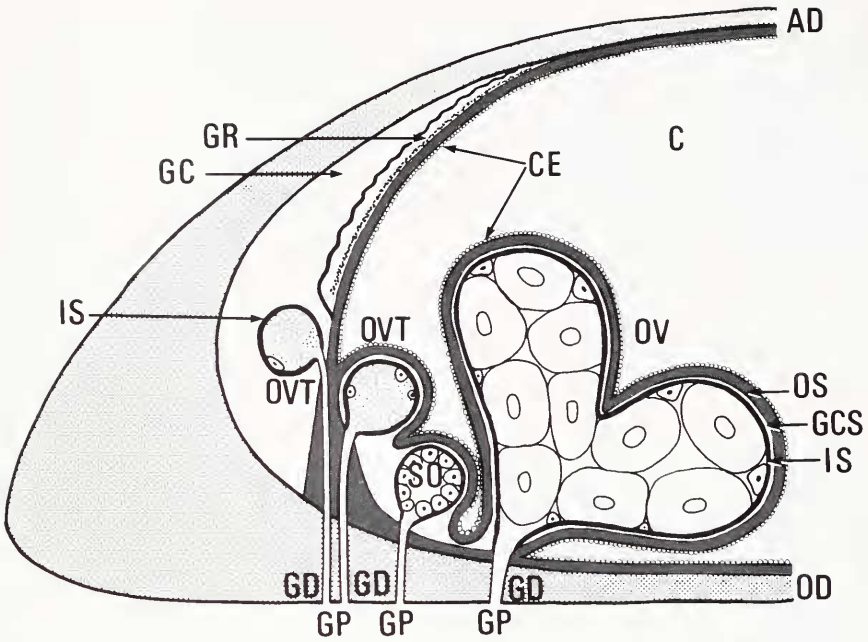

FIGURE 1. Diagrammatic representation of the gonad's alignment in a series of gonads during the breeding season of *Asterina minor*. The three types of gonads, ovary (OV), supernumerary ovary (SO), ovotestis (OVT) are arranged regularly from central (right) to marginal and oral (below) to aboral. The relationship of the gonads to the genital coelom are shown. AD, aboral side of the disk; C, coelomic cavity; CE, coelomic epithelium; GC, genital coelom; GCS, genital coelomic sinus; GD, gonoduct; GP, gonopore; GR, genital rachis; IS, inner sac; OD, oral side of the disk; OS, outer sac; OV, ovary; OVT, ovotestis; SO, supernumerary ovary.

the ovary and penetrates the body wall and opens in the gonopore. The oocytes reach more than 400 μm in diameter and have a large quantity of eosinophilic yolk. Small basophilic oocytes also occur in the spaces between the large eosinophilic oocytes (Fig. 2E). These two stages of oocytes are surrounded by follicle cells and are attached to the basal germinal membrane. Infrequently, gonial cells are also observed in the basal germinal membrane. The ovarian wall is composed of the inner and outer sacs, the interspace being the genital coelomic sinus, as described by Walker (1974) (Figs. 1, 2C, D). The outer sac consists of connective tissue and muscle, and is covered by a single layer of coelomic epithelium. The inner layer of the inner sac is the germinal epithelium.

A few ovotestes align at the most marginal and aboral region (Fig. 1, 2A, B). They are smaller than the ovary, but have independent gonoducts. The ovotestes form fully developed spermatogenic columns with spermatogonia, spermatocytes and spermatids, and sperm accumulating in the lumen of the gonads (Fig. 2F). In addition to these spermatogenic cells, small basophilic oocytes are frequently found in the ovotestes (Figs. 2F, G, 4F). The small oocytes are in contact with the basal germinal membrane and are enclosed by follicle cells. The number and the size of the small oocytes vary in different ovotestes within the individual as well as among different individuals, but the oocytes do not accumulate enough yolk to reach the maximum size. The ovotestes are of two types determined by their position: one type protruding into the coelomic cavity, surrounded by the inner and the outer sac (Fig. 1, right ovotestis, 2D, left ovotestis); the other type remaining in the

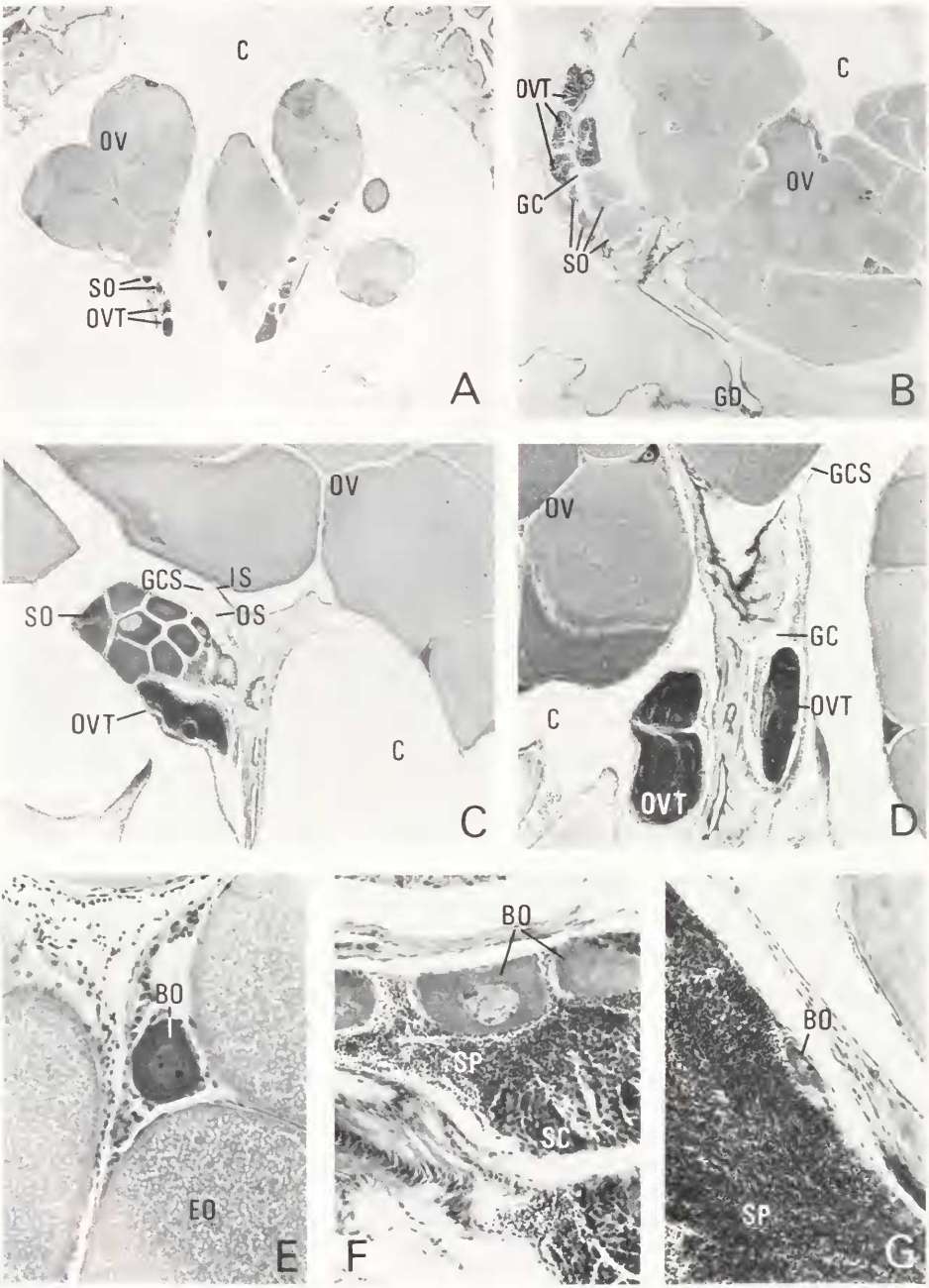

FIGURE 2. The alignment of the three types of gonads and their sexual cells.

2A. (May) Two series of gonads. Positions of the gonads are from central (upper) to marginal. The left series of gonads has an ovary (OV), two supernumerary ovaries (SO), and two ovotestes (OVT). ×25.

2B. (January) A growing ovary, four supernumerary ovaries, and three ovotestes are shown. The latter are in the genital coelom (GC). The gonoducts (GD) of all the gonads extend separately but are not shown in this section. ×25 (transverse section, others are horizontal).

2C. (May) A supernumerary ovary having small basophilic oocytes is lying between the mature

genital coelom, surrounded only by the inner sac (Fig. 1, left ovotestis, 2D, right ovotestis).

A variable number of the supernumerary ovaries with gonoducts occur between the ovary and the ovotestes (Figs. 1, 2A, B, C). Larger animals tend to have more than one supernumerary ovary (Table I). The supernumerary ovaries have fewer oocytes than the ovary, and infrequently have oocytes with yolk accumulated.

In each series of gonads, the ovary, the supernumerary ovaries, and the ovotestes align regularly from central to marginal and oral to aboral (Figs. 1, 2A, B).

Spawning

In Tokyo Bay, spawning of *Asterina minor* takes place in May. After spawning, the ovaries decrease in size, but small basophilic oocytes remain and non-germinal cells increase greatly in number (Fig. 3A).

By the time the ovotestes are ready to spawn, the gonads are filled with sperm (Fig. 2G). Following sperm release, the ovotestes reduce greatly in size, and the remaining small basophilic oocytes come into close proximity to each other (Fig. 3B). After spawning, the remaining sperm are gradually phagocytized by non-germinal cells and the spawned ovotestes look like supernumerary ovaries (Figs. 3C, D).

Formation of new ovotestes

During the breeding season or after spawning time, new gonads are formed. From the aboral haemal ring, two genital rachises extend downward in each interradial area (Figs. 1, 4A, B). Therefore, the genital rachis is more aborally positioned than the series of gonads. The germinal cells in the rachis begin to increase in number and swell out into the genital coelom (Figs. 3B, 4C, D). The thin layered, small gonads do not develop gonoducts nor oocytes but remain as lumps of germinal cells. However, thick layered, large gonads which are obviously ovotestes, acquire gonoducts and small basophilic oocytes (Fig. 4E). In November, the most developed gonads differentiate spermatogenic columns and sperm, and the enlargement of the gonads continues until the next breeding season (Figs. 4F, G).

In adults, a few new ovotestes are formed every year in the marginal and aboral position of each series of gonads. Their development differs within an individual as well as among different individuals.

Discussion

Gonads are formed from the genital rachis in echinoderms. Houk *et al.* (1980) studied the genital rachis in juvenile sea urchins and its gonad formation. In adult

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ovary and the ovotestis. In the ovary two sacs of gonadal wall, inner sac (IS) and outer sac (OS), with the intervening genital coelomic sinus (GCS) are shown. ×62.5.

2D. (May) A large mature ovary and the mature ovotestes are shown. The left ovotestis is surrounded by two sacs and the right one in the genital coelom is surrounded by the inner sac only. ×62.5 (compare with Fig. 1).

2E. (May) An ovary having large eosinophilic oocytes (EO) and small basophilic oocytes (BO). ×250.

2F. (April) Ovotestes with spermatogenic columns (SC), sperm (SP), and some small oocytes. The oocytes are surrounded by follicle cells. ×250.

2G. (May) The ovotestis filled with mature sperm and a small basophilic oocyte that is in contact with the basal germinal membrane. ×250.

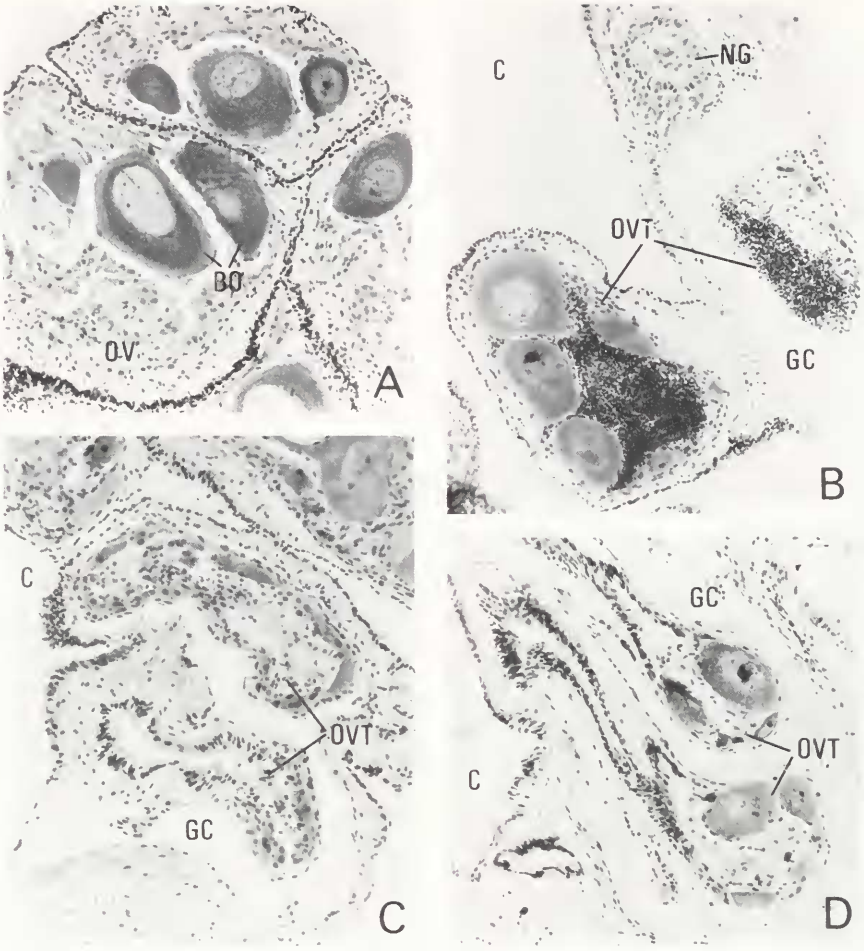

FIGURE 3. The spawned gonads.

3A. (June) Spawned and reduced ovary (OV) having small basophilic oocytes (BO) and a large number of non-germinal cells. ×250.

3B. (July) Ovotestes (OVT) releasing sperm. The oral (lower left) ovotestis having several small oocytes is surrounded by two sacs and projects into the coelomic cavity (C). The right ovotestis in the genital coelom (GC) having fewer oocytes is surrounded only by the inner sac. The aboral cell mass is a new gonad (NG). ×250 (transverse section, others are horizontal).

3C. (August) Two marginal and aboral ovotestes in the genital coelom. The upper ovotestis contains a few sperm which are being phagocytized by the non-germinal cells. ×250.

3D. (August) Two marginal and spawned ovotestes look like supernumerary ovaries. ×250.

sea urchins the rachis disappears. Adult ophiuroids have the genital rachis in the aboral haemal ring and the branches of the rachis form the gonads (Hyman, 1955). In the juvenile *Asterias rubens*, the genital rachis is formed in the aboral haemal ring with its 10 branches and establishes the gonads (Gemmill, 1914). On the contrary, in *Asterina minor*, the genital rachis remains until adult and forms ovotestes annually.

It is probable that the spawned ovotestes change into the supernumerary ovaries. During and after the breeding season, since there are very few gonial cells in most ovotestes, it is not conceivable that a large quantity of testicular tissue differentiates once more in the spawned ovotestes. Moreover, the oocytes in the ovotestes do not

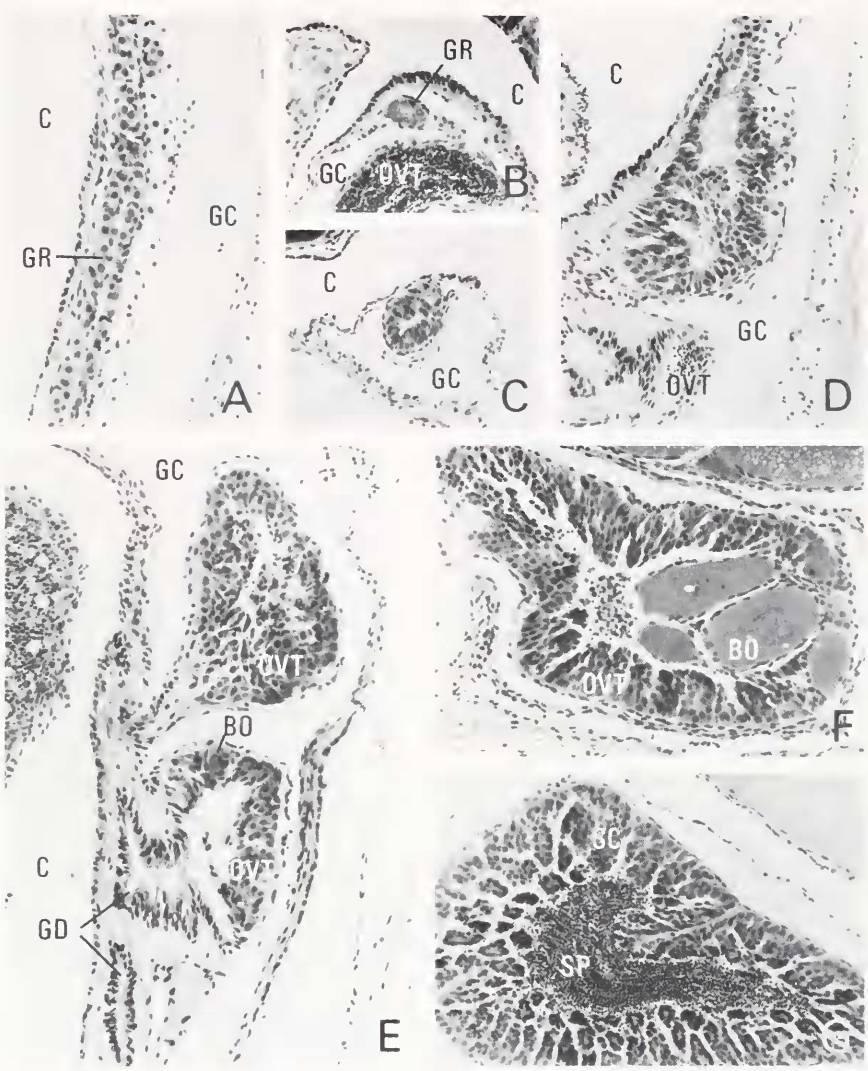

FIGURE 4. Formation of new gonads. A, D, E, and G are transverse and B, C, and F are horizontal.

4A. (May) A longitudinal section of the genital rachis (GR) which is adjacent to the wall of the genital coelom (GC). ×250.

4B. (May) The tubes of the germinal cells in the genital rachis are shown. The ovotestis (OVT) in the genital coelom is filled with sperm. ×250.

4C. (August) The germinal cells increase in number and swell out into the genital coelom. The new gonad has not formed its gonoduct yet. ×250.

4D. (July) The swollen part of the genital rachis. The oral (below) spawned ovotestis has a small number of relict sperm. ×250.

4E. (September) Two ovotestes having their own gonoducts (GD). A small basophilic oocyte (BO) is shown in the lower ovotestis. ×250.

4F. (November) An ovotestis having testicular tissue and small oocytes. Most of the oocytes are in contact with the basal germinal membrane. ×250.

4G. (January) An ovotestis having spermatogenic columns (SC) and sperm (SP). ×250.

mature. In the ovary, there are two stages of oocytes in the breeding season: maturing eosinophilic oocytes and small basophilic oocytes. Unpublished data show that the basophilic oocytes may be the source of the eosinophilic oocytes of the

next breeding season. If the spawned ovotestes underwent testicular tissue differentiation some time out of season, second year oocytes should be formed together with mature sperm in once spawned ovotestes. Examples in which about two years are required for oocyte development have been reported by Worley *et al.* (1977) and Chia (1968).

The observations suggest that in *Asterina minor*, the ovotestes are protandric, are formed annually, and after spawning may change into the supernumerary ovaries. The hermaphrodism of this species is maintained by the ovotestes and the ovaries.

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